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Short Communication

Evaluation of a "Chirasil-Val" capillary for the gas chromatography of volatile oil constituents, including sesquiterpenes in patchouli oil

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Abstract

The methyl-polysiloxane phase "Chirasil-Val" containing about 6% branched aliphatic side chains with L-valine in diamide linkage, has had its response assessed to various volatile oil constituents. Changes in relative retention times with temperature increase were distinctively large for camphor and fenchone. There was a greater spread of retention indices for seven solutes than on fully methyl polysiloxane, although less difference for another three solutes. Isothermally at 130°C, Chirasil-Val gave better resolution of the sesquiterpene hydrocarbon mix in patchouli oil than methyl polysiloxane, and could then be temperature programmed to evaluate patchoulol, etc. Carbowax 20M is a poor phase for patchouli analysis.

1. Introduction

This author has previously used some chirally selective cyclodextrin phases to identify constituents from volatile oils [1–3] and analyse oils of sweet fennel, mace [4] and dill [3]. It was now of interest to try for this a different chirally selective phase —a modified polysiloxane with branched aliphatic diamide sidechains, "Chirasil-Val". This was invented by Frank *et al.* in 1977 [5] for amino acid esters, and named and used a year later [6] for some sympathomimetic drugs and metabolites including ephedrine, penicillamine and L-DOPA. The phase has high thermal stability, unlike the modified cyclodextrins which are subject to thermal shock. It is depicted in Fig. 1, where the diamide-linked L-valine is attached to about every eighth silicon atom of

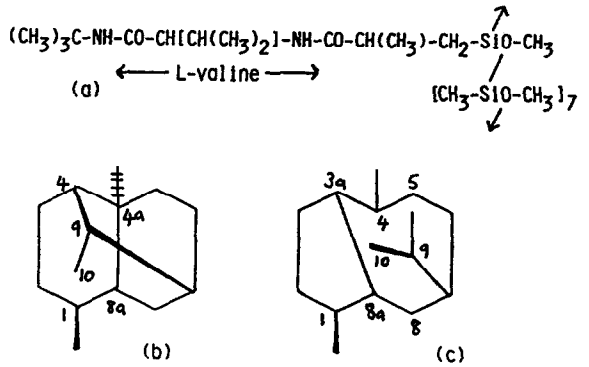

Fig. 1. (a) Chirasil-Val stationary phase [6]. (b) Patchoulol is saturated with 4-OH and 9-CH_3 groups. Seychellene is a 9–10 monoene with a 4-CH_3-group. (c) α-Bulnesene is a 3*a*–4,9–10 diene. α-Guaiene is a 3*a*–8*a*,9–10 diene. α-Gurjunene is a tricyclic (8–9)1–8*a* monoene. α-Patchoulene is a tricyclic (3*a*–9)4–5 monoene. β-Patchoulene is a tricyclic (4–9)3*a*–8*a* monoene.

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Table 1
Retention indices and relative retention times (linalol = 1.0) of various solutes on Chirasil-Val and other phases at given temperatures (°C)

Solute		Retention indices (*vs. n*-alkanes)								Relative retention times						
		Chirasil-Val							MPS[a]	Chirasil-Val						Chiraldex-A-DA [3]
		110°C — inc[b] →		170° ← inc —		140°C ← inc —				110°C — inc →		170° ← inc —		140°C — inc →		140°C
Caryophyllene	BH[c]			1523	29	1494	66		1428			3.59	0.03	3.56	0.05	3.61
Anethole	AE			1430	18	1412	142		1270			2.31	−0.05	2.36	1.36	*3.72*[d]
Cuminal	AC			1425	26	1399	172		*1227*			2.28	*0.09*	*2.19*	*1.03*	3.22
Borneol	BL			1395	14	1381	217		*1164*			1.97	−0.02	1.99	0.39	2.38
α-Terpineol	ML			1380	5	1375	190		1185			1.86	−0.08	1.94	0.24	2.18
Isoborneol	BL			1370	19	1351	194		*1157*			1.76	0.03	1.73		
4-Terpineol	ML	1328	18	1346	7	1339	164		*1175*	1.65	−0.05	1.60	−0.01	1.61	0.22	1.83
Estragole	AE	1291	38	1329	16	1313	130		1183	1.35	0.12	1.47	0.05	1.42	0.62	*2.04*
Camphor	BC	1271	64	*1335*	35	1300	164		1136	1.17	0.32	*1.49*	0.16	1.33	−0.07	1.26
Citronellal	NC	*1238*	28	1266	11	1255	118		1137	*0.98*	0.13	1.11	0.05	1.06	0.08	1.14
Linalol	NL	1246	−10	1236	−7	1243	151		1092	1.00		1.00		1.00		1.00
Fenchone	BC	1196	40	1236	17	1219	139		1080	0.74	0.26	1.00	0.11	0.89	−0.10	0.79
γ-Terpinene	MH	1110	17	1127	7	1120	63		1057	0.45	0.16	0.61	0.07	0.54	−0.01[e]	0.44[e]
Cineole	BE	1096							1027	0.41					0.03	0.44
p-Cymene	AH	1088							1020	0.39					−0.01	0.38
Limonene	MH	1078							*1030*	0.36					0.03	0.39

[a] Fully methyl polysiloxane phase, *e.g.* OV-101 [13]. No temperature given.
[b] Difference in values for a solute between adjacent columns, with increase (inc) direction shown by arrow. Note columns are not in order of temperature sequence.
[c] Information about the chemical nature of the solutes. A = Aromatic; B = bicyclic; C = ketone/aldehyde; E = ether; H = hydrocarbon; L = alcohol; M = monocyclic; N = acyclic.
[d] Numerical values out of descending sequence are italicized.
[e] These, and values below them in these columns at 110°C.

the otherwise methyl polysiloxane (about 6% of the side chains).

The important perfumery fixative patchouli oil is distilled from the leaves of *Pogostemon cablin* Benth. Its main constituents are sesquiterpene hydrocarbons and alcohols, the latter being mostly the tricyclic patchoulol [7] which forms about one-third of the oil. Another one-quarter or more is formed by two bicyclic hydrocarbons α-guaiene and α-bulnesene (synonym δ-guaiene) [8]. See Fig. 1 for formulae. Significant minor hydrocarbons include the tricyclic β-patchoulene, α-gurjunene [8] and seychellene [9]. α-Gurjunene is possibly a sign the patchouli oil has been adulterated with gurjun balsam [9], although other gurjunenes should then be found.

In the first gas chromatographic study of patchouli oil in 1962, Bates and Slagel [10] used a packed column of Carbowax 20M at 193°C and found (in retention time sequence) approximately 2% β-patchoulene, 21% α-guaiene, 21% α-bulnesene and 35% patchoulol. In 1967, Tsubaki *et al.* [8] used a similar polar phase capillary at 150°C for the hydrocarbons (!) only, obtaining β-patchoulene (12.8 min.) α-gurjunene (14.1 min), β-elemene (15.0 min), α-guaiene (16.0 min.), mixed caryophyllene (16.5 min.), mixed α-patchoulene, etc. (19.8 min.) and α-bulnesene (23.4 min.). α-Guaiene and α-bulnesene were the main constituents. In 1970, Henderson *et al.* [11] used a polar free fatty acid phase capillary to obtain the same sequence, but without any gurjunene or elemene directly from *Pogostemon* plant tissue. Since this, the gas chromatography of patchouli oil has been reviewed [12]. In the present work, a Chirasil-Val capillary was used for patchouli oil, after checking its performance with a number of volatile oil constituents used before [1–4]. Under the operating conditions used here, resolution of any enantiomers (if present) was unlikely.

2. Experimental

2.1. Apparatus

A Hewlett-Packard 5790A gas chromatograph was used, fitted with a capillary control unit, and a splitter injection port (split ratio 90:1 or more) and flame ionisation detector, both set at 215°C.

The Chirasil-Val capillary was purchased from Alltech (Deerfield, IL, USA) and was 25 m × 0.25 mm I.D. with film thickness given as 0.16 μm. Helium was the mobile phase, used at about 1 ml min^{-1}, and as "makeup" gas to the detector.

GC–MS apparatus used as an adjunct has been recorded previously [1].

2.2. Materials and methods

Solutes from various commercial sources were used [3]. The patchouli oil (Rivendell, Bunbury, Western Australia) was very dark brown, and 0.1 μl was injected from a microsyringe. Patchouli was run isothermally at 130°C for 8 min to elute the sesquiterpene hydrocarbons, then programmed up at 5°C min^{-1} to 190°C. Patchoulol emerged at 168°C. Single oil constituents were studied at 110°, 140° and 170°C. Trace residues from an "emptied" syringe were sufficient for the solutes. Holdup times of the solutes, obtained by extrapolating to methane the times for *n*-heptane and *n*-hexane on semi-logarithmic graph paper, were deducted from observed retention times.

3. Results and discussion

Results for various solutes compared with some literature values are given in Table 1 and some depicted in Fig. 2. Retention indices on Chirasil-Val usually increase with a rise in temperature, linalol being the exception. This gives a solute sequence "switch" with citronellal at about 120°C. It appears that fenchone-linalol also "crossover" at 170°C due to the declining values of the latter. Another "switch" occurs with camphor–estragole at about 160°C, due to the exceptionally rapid increase in camphor values (by +64 retention index units; other solutes only increase +17 to +40) going from 110°C to 170°C (see Table 1).

Camphor is a bicyclic ketone, and fenchone, which is another molecule of this type, increases by +40. However, a similar increase is shown by

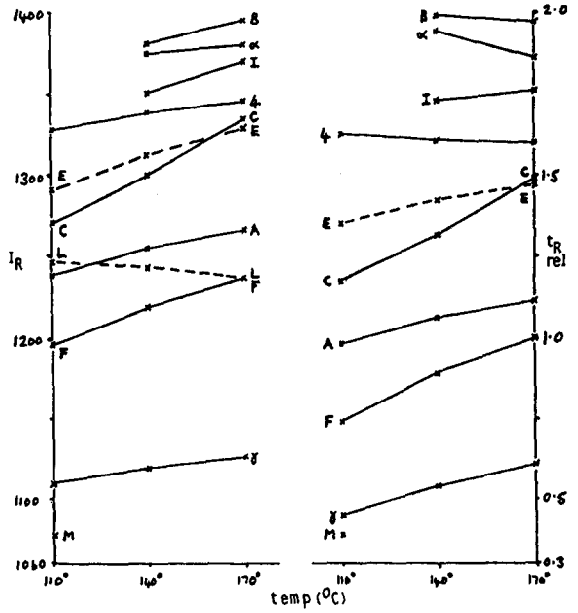

Fig. 2. Plots of retention indices (left) (I_R) and relative retention times (linalol = 1.00, right) ($t_{R,rel}$) for some solutes on Chirasil-Val phase. A = Citronellal; B = borneol; C = camphor; E = estragole; F = fenchone; I = isoborneol; L = linalol; M = limonene; α = α-terpineol; γ = γ-terpinene; 4 = 4-terpineol.

the aromatic estragole and other diverse solutes over 140°C to 170°C. But if relative retention times against linalol are examined, camphor and fenchone again exhibit considerable increase (+0.32 and +0.26 respectively) compared to a +0.14 average for the dissimilar estragole, citronellal and γ-terpinene from 110 to 170°C. 4-Terpineol gives a slight decrease; as do α-terpineol, borneol and anethole going from only 140 to 170°C.

Retention indices on fully methyl polysiloxane (MPS) are all lower [13] than those on Chirasil-Val at 140°C (Table 1), even for the hydrocarbon solutes (by about −65). Bicyclics borneol and isoborneol show the greatest increases going to Chirasil-Val at 140°C of +217 and +194 respectively, with other increases being +118 (citronellal) or more. The Chirasil-Val modified methyl polysiloxane exhibits a greater spread of indices for seven solutes (126, see Table 1) which are "condensed" over only 49 retention index units on the fully methyl polymer (citronellal to borneol). In contrast, the solutes cuminal–anethole–caryophyllene are expanded on MPS, with bigger gaps between their retention indices, than on Chirasil-Val (totals of 201 *vs.* 95, respectively). Comparing solute elution sequences on the two phases, borneol, isoborneol and 4-terpineol are "out of order", being eluted later from Chirasil-Val. Limonene, however, elutes earlier from this phase.

Relative retention times on Chirasil-Val were compared with other phases capable of enantiomer resolution from which they had been obtained previously —modified α-cyclodextrin capillaries [3]. "Chiraldex-A-TA" (trifluoracetyl, dipentyl) gave the closest resemblance, with half of fourteen solutes closely similar, particularly the bicyclics. However, this phase gave less satisfactory patchouli oil chromatograms than "Chiraldex A-DA", which showed five similar results. Chirasil-Val had considerably less affinity than A-DA (dipentyl, monohydroxy) for anethole, cuminal and estragole. Thus there is similar "selection" for terpenoids, but less retention of aromatics by Chirasil-Val. With these characteristics it was possible that this phase would be valuable for sesquiterpene oils.

It is difficult to see why bicyclic ketones should be "favoured" by Chirasil-Val with its branched aliphatic (six methyl groups) di-amides (see Fig. 1a). Perhaps their rigid "box-like" molecules fit well, unlike flat aromatics, between these special side chains which are only present on every eighth silicon atom of the polymer backbone? The polarity of Chirasil-Val is low, though higher than fully methyl polysiloxane (0.46 at 140°C compared to 0.27 respectively by "*c* ratio" [14]) both rising slightly with temperature increase. The *c* ratio (0.75[retention times of cuminal/caryophyllene]) of Chiraldex-A-DA at 140°C is higher still, 0.67, although it is the least polar of the three α-cyclodextrin modifications studied [3].

A "test" mixture of three commercially available, fairly pure, sesquiterpene hydrocarbons was used to evaluate Chirasil-Val as a phase for the analysis of volatile oils containing such substances. At 155 and 140°C, longifolene and caryophyllene emerged close together, but at

130°C they gave uncorrected retention times of 5.07 and 5.30 min, respectively, with humulene appearing at 6.10 min. The mix present in patchouli oil resolved quite well when programmed up after 8 min at 130°C (Fig. 3).

Peak identities for patchouli were assigned from a GC–MS run on a low polarity (MPS) DB-1 capillary. For the temperature programmed study of patchouli oil, Chirasil-Val gave better resolution of the complex mixture of sesquiterpenes than MPS although with the same retention sequence. The last main peak, patchoulol, was resolved from another constituent (Fig. 3) and shown to form only about 28.5% of the oil, and not "35%" (as on MPS). The earlier hydrocarbon peak of seychellene was well resolved from α-guaiene and reliably found to form about 7% of the oil. The very early β-patchoulene peak was seen to consist of two components, and only formed less than 2% of the oil. A 20M capillary also gave inferior resolution —high patchoulol and obviously impure peaks for α-bulnesene and α-guaiene (each about "18%") approaching early values found on this phase [10]. Chiraldex-A-DA also gave unsatisfactory values for α-bulnesene and α-guaiene.

It is logical that β-patchoulene elutes earlier from gas chromatographic phases than most other tricyclic mono-unsaturated sesquiterpene hydrocarbons. Its double bond is sterically hindered between two rings, and is tetra-substituted (Fig. 1c), allowing faster elution. In contrast, the exocyclic double-bond in seychellene is not hindered, with two hydrogen atoms at the outer end, and shows relatively long retention. Of the two dienes, α-guaiene has a sterically hindered double bond, and so elutes earlier than α-bulnesene.

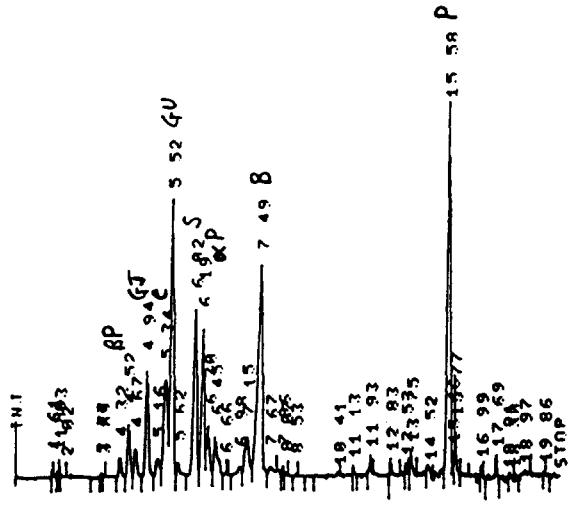

Fig. 3. Chromatogram of patchouli oil on Chirasil-Val capillary with chartspeed doubled between 4 and 8 min. Peak retention times printed in min. Peaks named from GC–MS runs on DB-1 and DB-23 with percentage identification *quality* from the computer library shown, together with the largest MS ions (*m/z*, in brackets). αP = α-Patchoulene *91* (93, 107, 135); βP = β-patchoulene *97* (161, 189, 119); B = α-bulnesene *99* (93, 107, 108); C = caryophyllene *99* (69, 93, 133); GJ = α-gurjunene *99* (204, 161, 189); GU = α-guaiene *99* (105, 147, 93). P = patchoulol peak at 168°C *97* (83, 98, 138, 222); S = seychellene —not in Wiley library (122 distinctive [8], 204). The area percentages of the identified peaks, in elution sequence, were β-patchoulene 1.7, α-gurjunene 3.9, caryophyllene 4.7, α-guainene 10.4, seychellene 6.9, α-patchoulene 6.4, α-bulnesene 12.0 and patchoulol 28.5.

4. Acknowledgements

The patchouli oil GC–MS runs were made by Mr. G.E. Chidlow and Mr. B. MacKinnon on DB-1 and DB-23 respectively. The observations on it using Chiraldex-A DA were made by Ms. S.C. Wong and Mr. D. Biancuzzo.

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